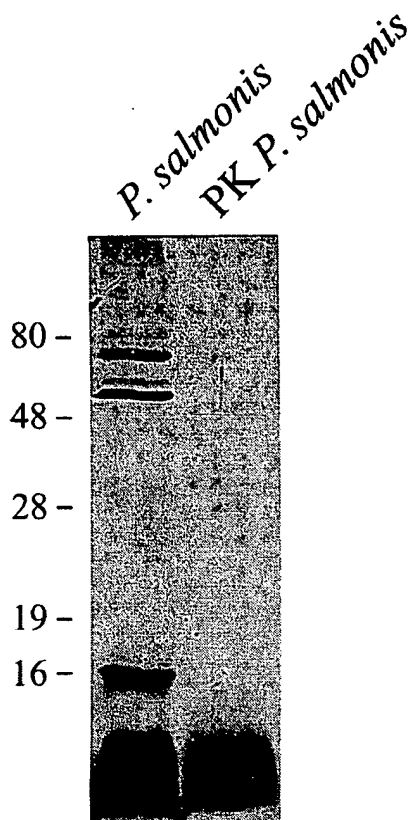


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Date of Deposit: February 18, 2004

Our Ref. No. 4616-67958
In re application of: Kuzyk et al.
For: VACCINES AND AGENTS FOR
INDUCING IMMUNITY IN FISH
AGAINST RICKETTSIAL DISEASES,
AND ASSOCIATED PREVENTATIVE
THERAPY
Sheet 1 of 11

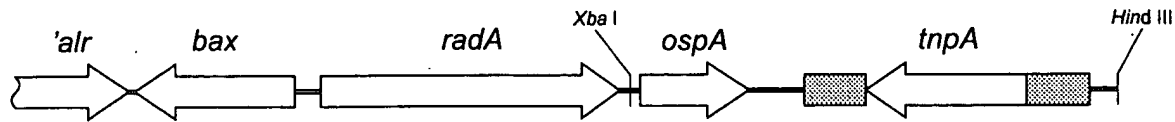
FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*



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FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAGGTAGTAGTCTAATTATTATCAGTGTGTTTTAGTTGGCTGTGCCCAGA
ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCCAGCTGTTTGG
TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAATTGGTTCATAAATC
GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGAAAAGGTAAAAGCAGGGCAAG
TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTGAGCCAGTGCCTACTTACCAGCGTTA
CAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTCAGCAAAAGGCGATGATTGCAGGGCAGAAG
CAAGAGATTTACGGCACTGCATGCCGGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

MNRGCLQGSSLI I I SVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVAMAIGGAVLGGLIGSKI
GQSMQDDKI KLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFOQKAMIAGQK
QEIYGTACRQPDGRWQVISTEK

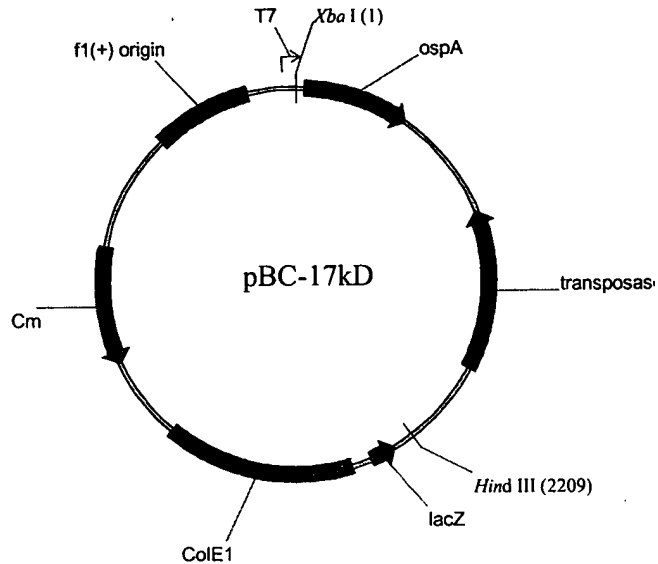
C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

Sequence alignment of *P. salmonis* and *R. prowazekii* proteins. The alignment shows two segments. The first segment (residues 1-40) shows high conservation between the two species. The second segment (residues 50-90) shows a deletion in *R. prowazekii* between residues 80 and 85. The alignment is presented in a grid format with residue numbers above and below the sequences.

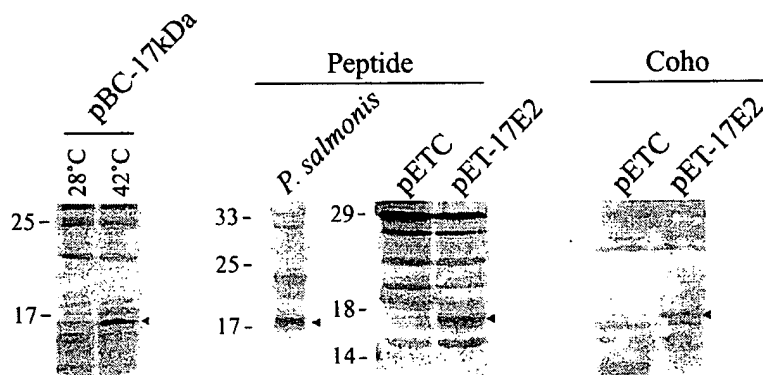
Residue	<i>P. salmonis</i>	<i>R. prowazekii</i>
1	M	M
2	N	K
3	R	L
4	G	L
5	C	S
6	L	K
7	S	I
8	L	M
9	I	I
10	I	A
11	S	A
12	V	A
13	---	S
14	---	M
15	F	L
16	V	L
17	G	C
18	C	A
19	---	N
20	---	G
21	O	S
22	N	G
23	F	M
24	S	N
25	R	K
26	O	G
27	V	I
28	G	T
29	A	L
30	A	L
31	T	L
32	G	L
33	A	G
34	V	A
35	V	G
36	G	G
37	V	A
38	G	G
39	V	A
40	G	L
41	---	S
42	---	G
43	---	A
44	---	L
45	---	S
46	---	G
47	---	A
48	---	L
49	---	S
50	F	F
51	G	G
52	K	C
53	S	K
54	G	G
55	R	L
56	V	V
57	A	G
58	M	L
59	A	G
60	I	G
61	G	G
62	A	G
63	I	G
64	L	G
65	G	G
66	L	G
67	L	G
68	L	G
69	L	G
70	L	G
71	L	G
72	L	G
73	L	G
74	L	G
75	L	G
76	L	G
77	L	G
78	L	G
79	L	G
80	L	G
81	L	G
82	L	G
83	L	G
84	L	G
85	L	G
86	L	G
87	L	G
88	L	G
89	L	G
90	L	G

FIGURE 3

A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.



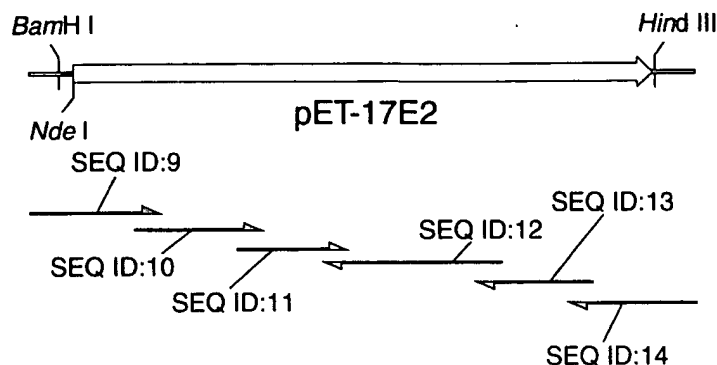
B. Western blot analysis of OspA expression.



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FIGURE 4.

A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.



B. Oligonucleotide #1 (SEQ ID:9)

CGCCAGGGTTTTCCCAGTCACGACGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
 ATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)

TGGGTTGCGCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
 CGGCCAGCTGTTTCGGTAAAGGCTCTGGTTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)

AAAGGCTCTGGTTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
 GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)

GTTCCACAGAGTAGCTGTTACCGGTGTCCGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTTC
 CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)

GGTGCCGTAGATTTCTGTTTCTGACCTGCGATCATGGCTTTCTGCTGAAATTCGCGGCAGTACTGCTGA
 CGGCGTTCCTGTTTGTGTAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)

CGTCCTCTCGTCTGGTCCGAATTCAGATAAGCTTATTTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
 GGCTGACGGCACGCGGTGCCGTAGATTTCCTGTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACT
 TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTTCGGTAA
 AGGCTCTGGTTCGTGTGTTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
 CAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCACTCTCTGGAAGAAAGTGAAGCCGCCAGGTTA
 CTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTGCGACCTACCAGCGTTACAA
 CAAACAGGAACGCCGTACGAGTACTGCCGCGAATTTACGAGAAAGCCATGATCGCAGGTCAGAAACAG
 GAAATCTACGGCACGCGGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVSMAGGAVLGGLIGSKIG
QSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQ
EIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

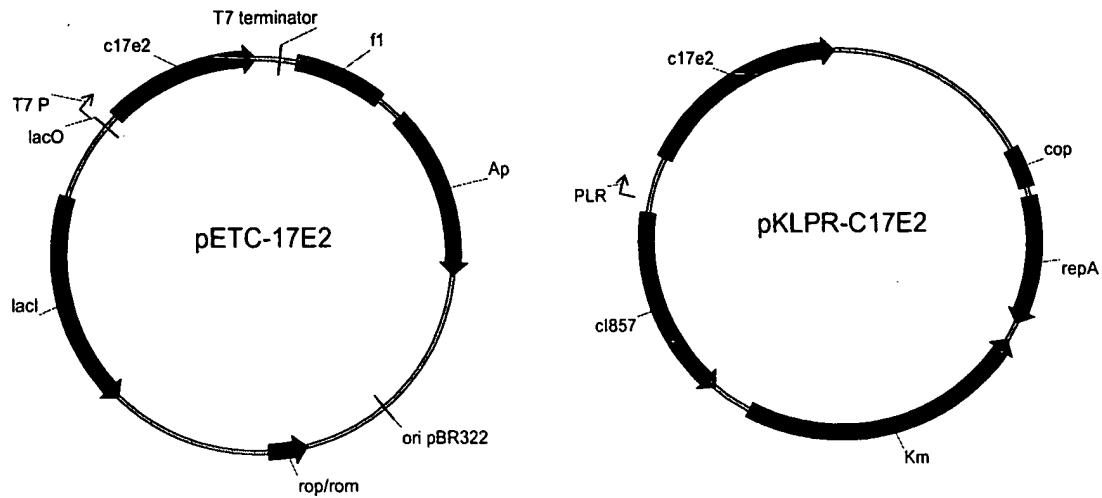
ATGTCAGTTGAATTCTACAACCTCTAACAAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAAGTGATGGTAC
ACAAGGACAAACTTTCTGGTGTGACCATGCTGGTGCAATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTTATCTCTGTTTTCTGGTGGGTTGCGCCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTC
GGTAAAGGCTCTGGTCGTGTGTGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACCACTCTCTGAAAAAGTGAAAGCCGGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAAACAGCTACTCTGTGGAACCGGTTTCGCACCTACCAGCGT
TACAACAAACAGGAACGCCGTGAGCAGTACTGCCGCGAATTTTTCAGCAGAAAGCCATGATCGCAGGTCAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

MSVEFYNSNKSQNTSITPIIKITNTSDSDLNLNDVKVRYYYTSDGTQGQTFWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDITYLDPSHMRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKGSGRVSMAGGAVLGGLIGSKIGQSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQR
YNKQERRQQYCREFQQKAMIAGQKQEIYGTACPQPDGRWQVISTEK

FIGURE 6

A. Expression vectors encoding the optimized *ospA* fusion constructs



B. SDS-PAGE analysis of C17E2 expression.

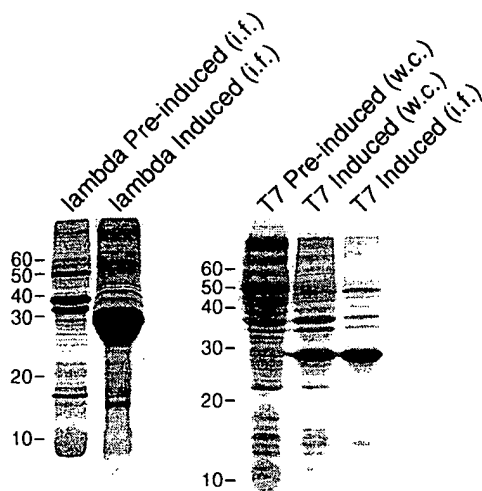


FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.

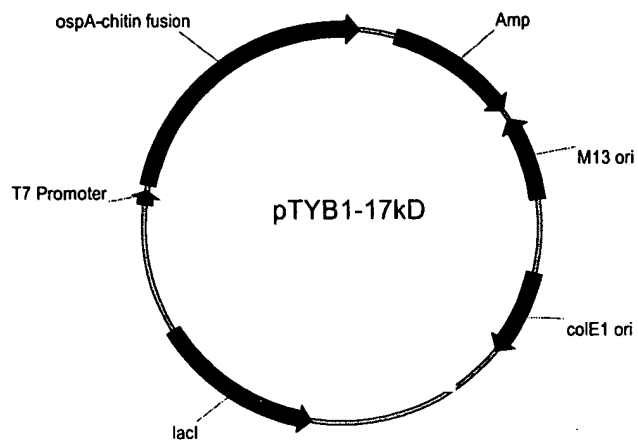
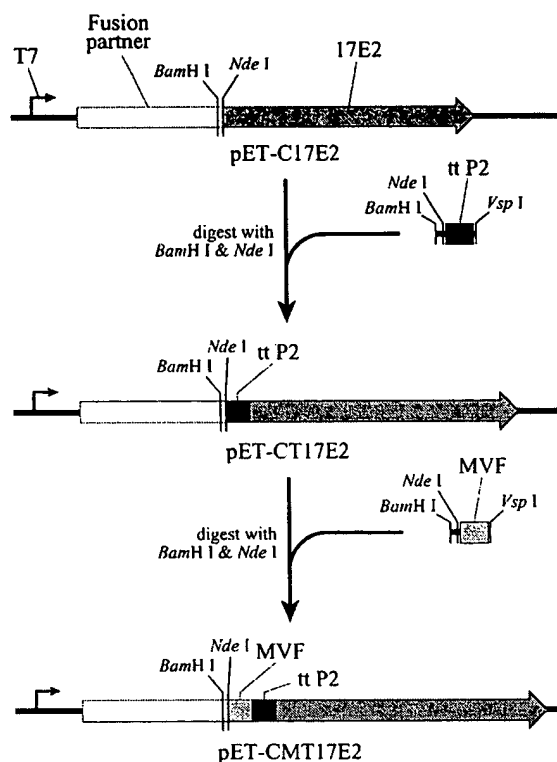


FIGURE 8

A. CLONING STRATEGY FOR OSP A TCE FUSION PROTEIN CONSTRUCTS.



B. (a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC
 GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT
 CGTCTGGAAGGCGTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVIVHRLEGV

FIGURE 9

**Coho salmon antibody titres against OspA-fusion protein
candidate vaccines.**

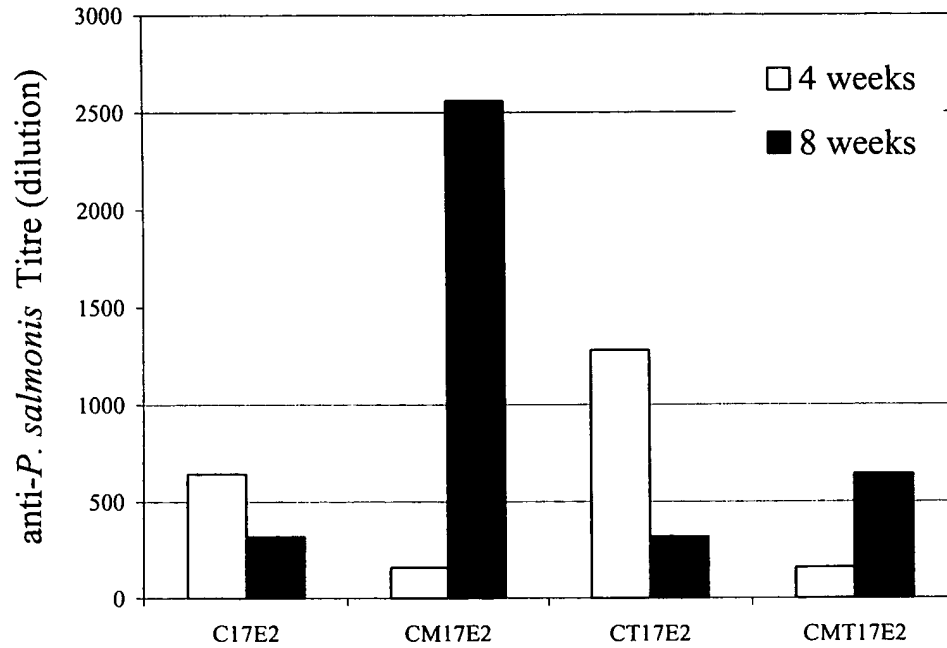


FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

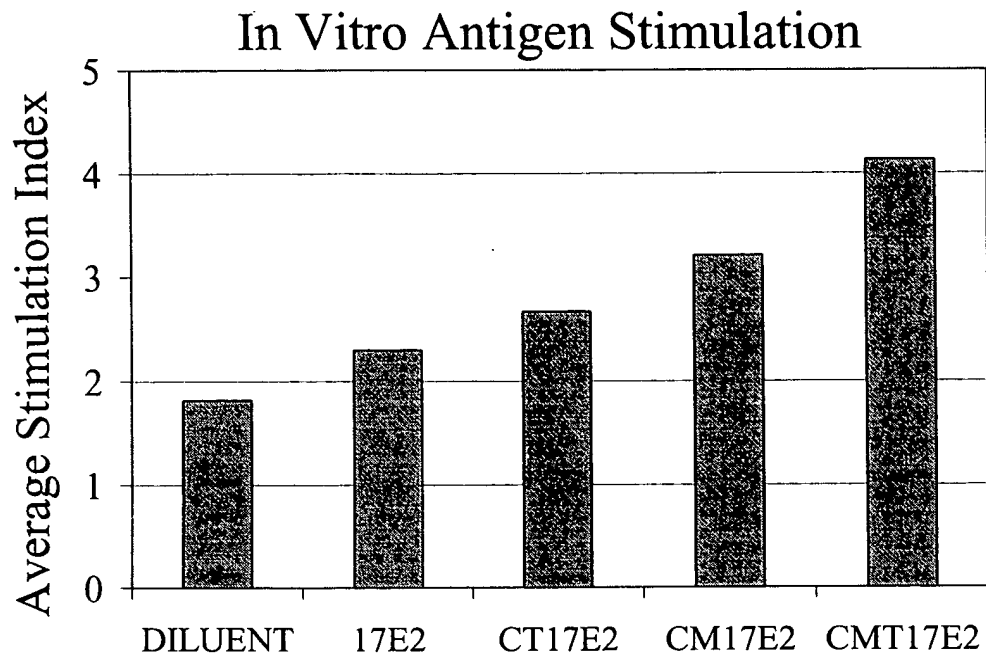


FIGURE 11

Vaccine trial in coho salmon of OspA fusion proteins.

